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Enrichment of Cadmium-Mediated Antibiotic-Resistant Bacteria in a Douglas-Fir 'Pseudotsuga menziesii' Litter Microcosm

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16. ABSTRACT

A set of Douglas-fir needle microcosms was amended with cadmium, acid, a combination of both, or neither. After 2 weeks of incubation, bacterial colony counts were made of litter homogenates inoculated onto agar media containing an antibiotic (streptomycin, chloromycetin, ampicillin, or gentamicin), cadmium, both, or neither. In all microcosms bacterial abundance was similar but the quality was very dissimilar Cadmium-treated microcosms had populations enriched for cadmium and gentamicin resistance and streptomycin and chloramphenicol sensitivity. Acid amendment had no consistent effect on the microcosm populations except that which could be attributed to the cadmium treatment amendment alone.

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Enrichment of Cadmium-Mediated Antibiotic-Resistant Bacteria in a Douglas-Fir (*Pseudotsuga menziesii*) Litter Microcosm

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A set of Douglas-fir needle litter microcosms was amended with cadmium, acid, a combination of both, or neither. After 2 weeks of incubation, bacterial colony counts were made of litter homogenates inoculated onto agar media containing an antibiotic (streptomycin, chloromycetin, ampicillin, or gentamicin), cadmium, both, or neither. In all microcosms bacterial abundance was similar but the quality was very dissimiliar. Cadmium-treated microcosms had populations enriched for cadmium and gentamicin resistance and streptomycin and chloramphenicol sensitivity. Acid amendment had no consistent effect on the microcosm populations except that which could be attributed to the cadmium treatment amendment alone.

Laboratory investigations have shown that many bacteria resistant to the effects of high concentrations of heavy metals are concomitantly resistant to several antibiotics (14). This is a current problem in hospitals where epidemiological investigations have shown that when metals are used to treat burns, there is an enrichment of metal-resistant bacteria that are also antibiotic resistant (2, 9, 19). This coresistance in other hospital situations is a relatively prevalent occurrence which is causing increased concern (6, 11, 12).

Outside the specialized confines of the hospital environment, the occurrence of metal and antibiotic coincidence in nature is being detected in aquatic habitats (5) where there are high concentrations of heavy metals, e.g., in the sediments of New York Bight (7, 21), the guts of fish living in heavy metal-polluted rivers (8), and sewage-polluted river water (22).

To the best of my knowledge the development of antibiotic-resistant soil bacteria exposed to heavy metals in the soil has not been reported, although materials containing heavy metals are being added to litter and soil systems in the forms of fly ash from smelters (17) and coal-fired power plants (13), automobile exhaust (4), chemical fertilizer application (18, 23), and the recent potentially very serious route, metals in sewage and industrial sludges that are applied to crop land (3, 20).

The experiment described here concerns the enumeration of antibiotic-resistant bacteria enriched in litter microcosms amended with cadmium, acid, both, or neither.

MATERIALS AND METHODS

Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) needle litter (approximate pH of 5.0) was air-dried and ground in a Wiley mill to pass a 2-mm mesh screen. Twenty-one grams (1.5 cm deep) of this material was placed in eight 1-quart Mason jars. Pairs of jars were amended with 39 ml of distilled water or 10^{-2} M HCl, either singly or in combination with 2 mM $CdCl_2$. Five milliliters of a litter inoculum (supernatant from 10 g of litter homogenized in 1 liter of distilled water which was allowed to settle for approximately 5 min) was added to each microcosm to bring the litter moisture to 70% of its water holding capacity. Every 2 or 3 days, depending upon litter respiratory activity, the airtight jars were opened and flushed with air, and a carbon dioxide trap (1-ounce [ca. 29.6-ml] screw-cap bottle containing 10 ml of 0.6 N NaOH) was replaced. After 6 weeks of incubation at 20°C in the dark, the contents of the duplicate microcosms were mixed, and 10 g of this material was homogenized in 100 ml of sterile buffered dilution water (1) in a Waring blender for 4 min. A 10-fold dilution series of each homogenate was prepared in sterile buffered dilution water and 0.1 ml of each dilution was inoculated onto, and spread over the surface of, air-dried 0.08% (wt/vol) nutrient broth (Difco) solidified with 1.5% (wt/vol) agar (Difco) plates containing 20 μg of chloramphenicol, dihydrostreptomycin sulfate, gentamicin sulfate, or ampicillin per ml, and/or in a 5 mM cadmium chloride solution, or none of these (control). Bacterial colony counts were made on these plates after 14 days of incubation at 20°C.

RESULTS AND DISCUSSION

The quantity of bacteria supported in the cadmium-treated litter (51.7×10^7 colony-forming units [CFU]/g) was essentially the same as

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the control litter (56.2×10^7 CFU/g) (Table 1). The quality of the two populations was markedly different. Only about 1% (74.0×10^5 CFU/g) of the control litter organisms were cadmium resistant, while almost 100% (56.3×10^7 CFU/g) were resistant in the cadmium treatment. No differentiation between cadmium-resistant selection, or "training" in the Pickett and Dean (15, 16) sense, can be ascribed to this observation.

When the antibiotics chloromycetin and especially streptomycin were added to the counting medium, it was evident that the microcosm with the cadmium-treated litter supported populations that were more sensitive to these agents (6 and 0.05% compared to the control medium count, respectively) than the control microcosm treatment (53 and 4.5% compared to the control medium count, respectively). Conversely, outgrowth from the gentamicin-amended culture media showed that there was a selection of bacteria that was more resistent to gentamicin in the cadmium-treated microcosm compared to the control microcosm (i.e., 27 and 6.6%, respec-

TABLE 1. *Colony counts of bacteria grown on culture media amended with antibiotic, cadmium, both, or neither, using inocula from identically prepared and incubated Douglas-fir (Pseudotsuga menziesii) needle litter microcosms amended with cadmium and/or hydrochloric acid*

Culture medium amendment	Microcosm amendment		Bacteria (CFU/g)	Control medium counts (%)	Cadmium medium counts (%)
	Cadmium[a]	Acid pH[b]			
Control	−	5.6	56.2×10^7	NA[c]	NA
	−	2.0	40.3×10^7	NA	NA
	+	5.6	51.7×10^7	NA	NA
	+	2.0	132.0×10^7	NA	NA
Cadmium[d]	−	5.6	74.0×10^5	1.3	NA
	−	2.0	28.3×10^5	0.7	NA
	+	5.6	56.3×10^7	109	NA
	+	2.0	81.7×10^7	62	NA
Chloromycetin[e]	−	5.6	29.7×10^7	53	NA
	−	2.0	14.0×10^7	35	NA
	+	5.6	28.7×10^6	6	NA
	+	2.0	8.7×10^7	7	NA
Chloromycetin + cadmium	−	5.6	7.0×10^6	NA	94
	−	2.0	13.0×10^5	NA	46
	+	5.6	4.0×10^6	NA	1
	+	2.0	7.7×10^5	NA	1
Streptomycin	−	5.6	25.0×10^6	4.5	NA
	−	2.0	16.3×10^6	4.0	NA
	+	5.6	2.7×10^5	0.05	NA
	+	2.0	4.0×10^5	0.03	NA
Streptomycin + cadmium	−	5.6	43.3×10^4	NA	6
	−	2.0	6.3×10^4	NA	2
	+	5.6	9.0×10^3	NA	0.002
	+	2.0	4.7×10^4	NA	0.006
Gentamicin	−	5.6	37.0×10^6	6.6	NA
	−	2.0	22.7×10^6	5.6	NA
	+	5.6	14.0×10^7	27.1	NA
	+	2.0	75.3×10^7	57.1	NA
Gentamicin + cadmium	−	5.6	21.7×10^5	NA	29
	−	2.0	15.3×10^5	NA	54
	+	5.6	13.3×10^7	NA	24
	+	2.0	20.7×10^7	NA	25
Ampicillin	−	5.6	13.2×10^7	23.5	NA
	−	2.0	13.7×10^7	34.0	NA
	+	5.6	21.7×10^7	42.0	NA
	+	2.0	24.3×10^6	1.8	NA
Ampicillin + cadmium	−	5.6	62.3×10^5	NA	84
	−	2.0	64.3×10^5	NA	227
	+	5.6	10.0×10^7	NA	18
	+	2.0	4.0×10^6	NA	0.5

[a] $CdCl_2$ (10 mM) in solution to 70% water holding capacity.
[b] pH of HCl solution added to microcosm to 70% water holding capacity.
[c] NA, Not applicable.
[d] $CdCl_2$ (5 mM)/liter.
[e] Antibiotic concentrations were 20 μg/ml.

tively). No significant trend in ampicillin resistance was discernible. Also, addition of cadmium to the culture medium did not change any of the foregoing trends.

The addition of hydrochloric acid as an acid rain similator to the litter had no consistent effect on the development of antibiotic-resistant populations beyond that attributed to cadmium alone. At lower concentrations of cadmium and substrates with a increased acidity and/or buffering capacity, one might observe a different result.

From this preliminary experiment, it is clear that cadmium added to a mixed population litter system enriches for bacteria that are both resistant to cadmium inhibition and are, more or less, resistant to antibiotic inhibition, depending on the antibiotic. The molecular mechanisms for both cadmium (10) and antibiotic (24) action have been described for some organisms, but in mixed populations it is difficult to explain these results in terms of those mechanisms, particularly when gentamicin-resistant bacteria are enriched and streptomycin numbers are depressed by cadmium amendment, yet both antibiotics are thought to have a similar action mechanism.

This cursory investigation indicates that further research is needed in specific areas of metals-mediated antibiotic resistance in soil systems: (i) a repeated and extended set of microcosm experiments using other metals and soils, (ii) a determination of the prevalence of antibiotic/metals resistance in soil populations around metal-polluted sources, (iii) a determination of the transducibility of antibiotic resistance from soil bacteria to human inhabiting forms, (iv) an epidemiological investigation corrolating soil contamination by metals and clinical cases of the co-occurrence of metals and antibiotic-resistant bacteria, and (v) the determination of how this phenomenon may effect general rhizosphere and particularly rhizosphere phytopathogenic bacterial populations.

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